Journal of Chromatography, 369 (1986) 265–268
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 19 000

Letter to the Editor

Comment on the observation of enhanced peak responses due to solvent interactions in high-performance liquid chromatography

Sir,

Perlman and Kirschbaum[1] have reported recently that ultraviolet detector response in liquid chromatography is dependent upon the solvent used for the analyte and the analyte's ability to form intramolecular hydrogen bonds. However, a number of points arise from their discussion that require comment.

(1) It is unfortunate that the paper does not supply sufficient experimental detail to facilitate easy repetition of their work. Thus no information is provided concerning the actual columns used, their dimensions or the specific packing and its particle size. Additionally, the details on solution concentrations are not fully explained.

(2) There appear to be more errors in the text than are consistent with simple typographical mistakes. Reference is made to the increase in areas *and* heights that occurs as solvent polarity increases yet the order quoted (water to methanol to ethanol) represents a decrease in polarity. Furthermore, Fig. 1 shows a decreasc in height for the UV detected peaks going from water to ethanol and, if the area units are correct on the figure, a decrease in area. Simple mensuration of the peaks, assuming that they have been plotted at the same sensitivity, clearly shows that, in contrast to the published figure, areas are increasing. It is extremely difficult to judge the refractive index detector data since the positive going peak due to the solute suffers from interference from a negative going (solvent?) peak. However, the published figures on the diagram indicate a simultaneous reduction in both height and area.

(3) The proposition that a peak detected at 270 nm is directly due to ethanol is surely worthy of a much more detailed investigation than is evident in this paper.

(4) Fig. 4, which shows the responses for the disulphonamide of bendroflumethazide, surely contradicts the hypothesis presented. Assuming identical weights were used, the areas are sufficiently close (a 9% difference) that the difference could be accounted for by the difficulty in accurately integrating such a broad peak: no mention is made of how peak mensuration was carried out. The peak areas are thus approximately constant and height response has dropped, consistent with reduced chromatographic efficiency.

(5) The comment that peak retention times "often are identical" is too imprecise in a study of this nature to be valuable and warrents a more detailed investigation. Three of the solutes described (captopril, nadalol and methyl *para*-hydroxybenzoate) have thus been re-investigated since, if the hypothesis presented can be substantiated, it follows that a method of probing intramolecular hydrogen bonding becomes available. All separations were carried out using a Hewlett-Packard Model 1090A liquid chromatograph, equipped with a diode-array detector and on-board

TABLE I

DETECTOR RESPONSES FOR CAPTOPRIL, NADALOL AND METHYL *para*-HYDROXYBENZOATE SOLUTIONS

Conditions: 11 cm × 4.6 mm I.D. Partisphere C_{18} column eluted with methanol–water–orthophosphoric acid (50:50:1); 5 μl was injected.

Solute	*Solvent*	*Retention time (min)*	*k'*	*Detection (nm)*	*Area (ma.u. s)*	*Height (ma.u.)*
Captopril	Water	1.088	0.96	220 ± 10	37.35 C.V. 0.6%	10.10 C.V. 0.6%
	Methanol	1.078	0.94		38.24 C.V. 1.2%	9.41 C.V. 0.7%
Nadalol	Water	2.851	4.13	270 ± 10	11.44 C.V. 0.5%	1.007 C.V. 0.6%
	Methanol	2.831	4.09		12.03 C.V. 1.7%	1.013 C.V. 1.2%
Methyl *p*-hydroxy-benzoate	Water	1.338	1.41	270 ± 10	285.2 C.V. 0.5%	89.2 C.V. 0.6%
	Methanol	1.329	1.39		299.7 C.V. 2.0%	83.1 C.V. 1.1%

DPU integrator, at 40°C. Two columns were used, an 11 cm × 4.6 mm I.D. stainless-steel column packed with 5-μm Partisphere C_{18} (Whatman) and a 4.5 cm × 4.6 mm I.D. stainless-steel column containing 5-μm Ultrasphere ODS (Beckman). Mobile phases comprised methanol and water with 1% added orthophosphoric acid.

Table I shows the chromatographic data obtained with 5-μl injections of solutions of the three solutes in either methanol or water solution. The mobile phase was 50% methanol in water with 1% orthophosphoric acid and the flow-rate was 1.5 ml min^{-1}. Peak areas and heights are quoted in ma.u. s or ma.u., respectively, and have been adjusted to account for the actual weight of solute in solution (0.5 to 0.8 mg ml^{-1}). The chromatographic system was equilibrated until retention time coefficients of variation were below 0.1%. In contrast to the results presented earlier[1], it is seen that, for solutes dissolved in water, retention times are significantly increased compared to those in methanol and, whilst heights are slightly increased, areas stay constant or decrease. While the absolute changes in areas and heights are within experimental error, the area–height relationship changes are significant—particularly for captopril and methyl *para*-hydroxybenzoate. These solutes represent one each from the hydrogen bonding and non-hydrogen bonding groups.

The peak response phenomena could also be produced with the shorter Ultrasphere column. A mobile phase of methanol–water–orthophosphoric acid (40:60:1) was used with a flow-rate of 1 ml min^{-1}. As before, 5-μl injections of the same solutions of captopril were made. In water retention times, areas and heights for captopril solutions were 1.255 min, 50.9 ma.u. s (C.V. 1.0%) and 5.98 ma.u. (C.V. 1.7%), respectively. In methanol values were 1.230 min, 50.5 ma.u. s (C.V. 1.5%) and 6.18 ma.u. (C.V. 0.5%), respectively. There are no significant differences in peak areas or heights although retention times have changed as before.

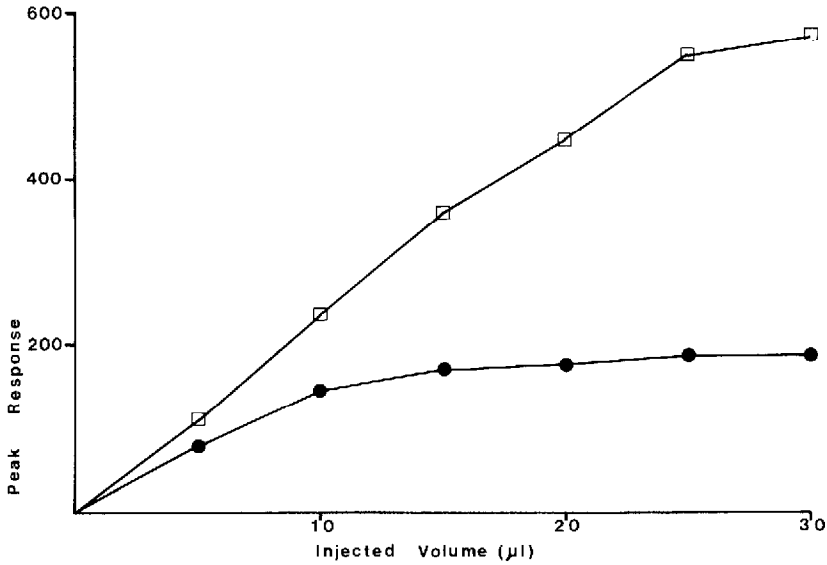

Fig. 1. Peak area and height responses for solution of captopril in methanol (0.8 mg ml^{-1}). A 4.5 cm × 4.6 mm Ultrasphere ODS column was eluted with methanol–water–orthophosphoric acid (40:60:1) at 1 ml min^{-1}. (□) Area, ma.u. s × 0.1; (●) height, ma.u.

To investigate the effect of mass of compound injected, injections of 5 to 30 µl were made using captopril in methanol. Linearity is lost above 10 µl and detector response now starts to follow the pattern described by Perlman and Kirschbaum[1]. Fig. 1 shows peak response as a function of injected volume while Fig. 2 compares a 25-µl injection of a solution of captopril in water with a 25-µl injection using methanol as solvent. The areas of the peaks are essentially identical.

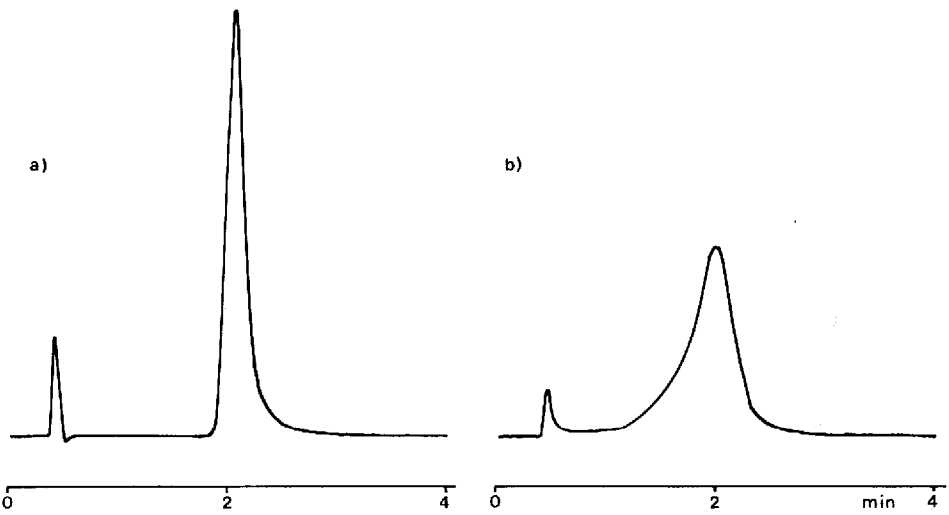

Fig. 2. Comparison of 25-µl injections of captopril solution in (a) water and (b) methanol. Conditions as for Fig. 1.

In conclusion, the proposition that intramolecular hydrogen bonding can affect peak response is a hypothesis that should be of interest to all chromatographers. Unfortunately, the original data presented cannot be considered to substantiate the hypothesis and it has proved impossible to reproduce the phenomena. May I suggest that this a subject for a more carefully controlled study which must include greater experimental detail and an investigation to ensure that instrumental artefacts are not influencing the conclusions?

ACKNOWLEDGEMENT

The interest of Mr. P. F. Wadsworth and Dr. D. V. Bowen of this department in this work is gratefully acknowledged.

Analytical Chemistry Department, Pfizer Central Research, Sandwich, Kent CT13 9NJ (U.K.) J. C. BERRIDGE

REFERENCE

1 S. Perlman and J. J. Kirschbaum, *J. Chromatogr.*, 357 (1986) 39.

(Received May 27th, 1986)